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Integrative taxonomic evidence for a new species of genus *Gracixalus* (Anura, Rhacophoridae) from Mao'er Mountain, Guangxi, China

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Abstract

It has been shown that the taxonomy of *Gracixalus jinxiuensis* species complex is very confusing. In this study, we tested for the taxonomy of *Gracixalus* species from Mao'er Mountain, Guangxi, China, which was once recorded as *G. jinxiuensis*, based on molecular, morphological, and bioacoustics evidence. Phylogenetic analyses based on mitochondrial 16S rRNA sequences indicated that the samples from Mao'er Mountain form a distinct lineage closely related to *G. jinxiuensis*, *G. huaping*, and *G. weii*, and they were assigned into an independent operational taxonomic unit by analysis of species delimitation. Morphologically, the lineage from Mao'er Mountain can be distinguished from known congeners by a combination of the following characters: SVL 27.5–33.2 mm in males and 38.1–39.7 mm in females; dorsal surface beige to brown, rough with a few tubercles on dorsum; internal vocal sac; linea masculina absent; nuptial pads present on fingers I and II; tibiotarsal articulation reaching center of eye; heels overlapping when legs at right angle to body; and white tubercles on temporal region, edge of upper and lower jaw, flank, and dorsal surface of limbs. In addition, bioacoustics analyses showed that the advertisement calls of the population from Mao'er Mountain also differ from advertisement calls of other *Gracixalus* species. Therefore, we officially described the lineage from Mao'er Mountain as a new species and named it as *Gracixalus liusanjieae* **sp. nov.** herein. Including the new species described in this study, now the genus *Gracixalus* contains 23 species, 12 of which are distributed in China with six known in Guangxi.

Key Words

Bioacoustics analysis, *Gracixalus jinxiuensis*, species complex, 16S rRNA, species delimitation

Introduction

The genus *Gracixalus* Delorme, Dubois, Grosjean & Ohler, 2005 has a wide distribution in the mountain forests of Thailand, Myanmar, Laos, Vietnam, India, and southern and southwestern China (Frost 2024). This genus is characterized by having small to medium body size, intercalary cartilage between terminal and penultimate phalanges of digits present, tips of digits enlarged to discs bearing circum-marginal grooves, vomerine teeth absent, inner (first and second) and outer (third and fourth) fingers not opposable, and an inversed Y-shaped dark brown marking on the dorsum (Fei 1999; Yu et al. 2019; Tran et al. 2023). Currently, the genus *Gracixalus* contains 22 species and more than half of them were described from southern China and Vietnam in the last decade based on morphological and molecular data (e.g. Liu et al. 2025; Luo et al. 2025), implying that the species diversity of the

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genus *Gracixalus* likely remains underestimated owing to conserved morphology. As of now, 11 species of the genus *Gracixalus* have been recorded from China and five are distributed in Guangxi, namely *G. gracilipes* (Bourret, 1937), *G. jinxiuensis* (Hu, 1978), *G. nonggangensis* Mo, Zhang, Luo, Zhou & Chen, 2013, *G. tianlinensis* Chen, Bei, Liao, Zhou & Mo, 2018, and *G. huaping* Luo, Zhang, Pan & Yu, 2025.

Mao'er Mountain, a part of Yuechengling Mountain range of Nanling Mountain system, is located in northeastern Guangxi and is famous for the headstream of Lijiang River and the top peak of South China. Its elevations range from 280 m to 2142 m a.s.l and it is characterized by rugged terrain with deep valleys and high mountains. The extreme topographic relief, together with its wide altitudinal range, have produced extreme differences in climate. The diverse climate and topography of the mountain have resulted in extremely high levels of biodiversity. Currently, according to Jiang et al. (2006), Mo et al. (2014) and AmphibiaChina (2024), more than 40 amphibian species including *G. jinxiuensis* are known from Mao'er Mountain.

Gracixalus jinxiuensis was originally described from Dayao Mountain, Jinxiu, Guangxi, China (Hu et al. 1978) and was once recorded widely from China (Guangxi, Hunan, Yunnan), Vietnam, and Laos. However, it has been revealed by studies in recent years that misidentifications were involved in this species and some records of this species actually belong to other species (e.g. Yu et al. 2019; Luo et al. 2025), indicating that the taxonomic status of other records of *G. jinxiuensis* also needs further investigation.

During recent fieldwork in Maoershan National Natural Reserve, Guilin, Guangxi, we collected six specimens of a small-sized tree frog resembling *G. jinxiuensis*. We conducted phylogenetic analyses, morphological comparison, and bioacoustics analyses to ascertain their identity. Phylogenetic analyses recovered these specimens as a distinct lineage of genus *Gracixalus*, and morphological examination and bioacoustics analyses demonstrated that this lineage is distinguished from known members of the genus *Gracixalus* by a series of characters. Herein, we officially describe the lineage consisted of samples from Mao'er Mountain as a new species of *Gracixalus*.

Materials and methods

Sampling

This study was carried out in accordance with the ethical guidelines issued by the Ethics Committee of Guangxi Normal University. Field surveys were conducted in April 2024, August 2024, and April 2025 under the permissions of Maoershan National Natural Reserve, and a total of six specimens of the genus *Gracixalus* were collected from Mao'er Mountain during the surveys (Fig. 1). Specimens were photographed, euthanized, fixed, and then stored in 75% ethanol. Liver tissues were preserved in 99% ethanol. Specimens were deposited at Guangxi Normal University (**GXNU**).

Phylogenetic analyses and species delineation

Genomic DNA was extracted from the collected liver tissue samples using standard phenol-chloroform protocols. We amplified and sequenced a partial fragment of mitochondrial 16S ribosomal RNA gene (**16S rRNA**) using the primer pair L2188 (Matsui et al. 2006)/16H1 (Hedges 1994) and the protocol of Yu et al. (2010). All new sequences have been deposited in GenBank under accession Nos. PV746479–PV746484 (Table 1). Homologous sequences of known species of *Gracixalus* were retrieved from GenBank.

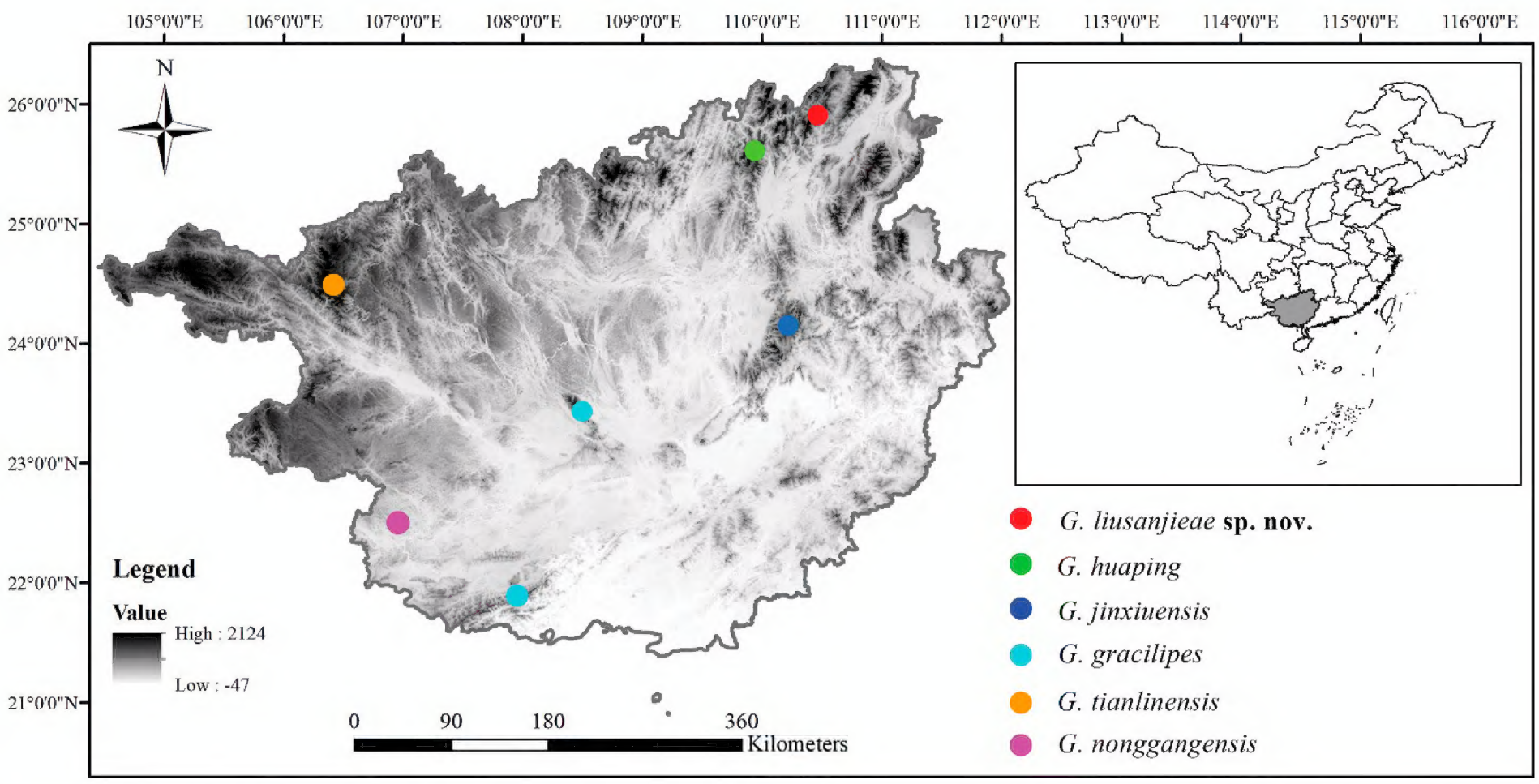

Figure 1. The distribution of *Gracixalus* species in Guangxi, China. The red circle represents the type locality of the new species (Mao'er Mountain). The map was produced using ArcMap v.10.8.1.

Table 1. Species used for phylogenetic analyses in this study.

Species	Locality	Voucher no.	GenBank no.	References
Rhacophorus borneensis	Sabah, Malaysia	BORN 22410	AB781693	Matsui et al. (2013)
Kurixalus idiootocus	Taiwan, China	KUHE 12979	AB933306	Nguyen et al. (2014)
Gracixalus yunnanensis	Bada, Menghai, Yunnan, China	KIZ 20160216	MK234877	Yu et al. (2019)
Gracixalus yunnanensis	Xuelin, Lancang, Yunnan, China	KIZ 20160222	MK234878	Yu et al. (2019)
Gracixalus yunnanensis	Fudong, Lancang, Yunnan, China	KIZ 20160226	MK234880	Yu et al. (2019)
Gracixalus yunnanensis	Fazhanhe, Lancang, Yunnan, China	KIZ 20160228	MK234881	Yu et al. (2019)
Gracixalus sapaensis	Lao Cai, Vietnam	CIB XM-439	GQ285670	Li et al. (2009)
Gracixalus sapaensis	Lao Cai, Vietnam	KUHE 46401	LC011938	Matsui et al. (2015)
Gracixalus sapaensis	Lai Chau, Vietnam	IEBR 2351	EU871425	Nguyen et al. (2008)
Gracixalus sapaensis	Lao Cai, Vietnam	MNHN 1999.5961	AY880503	Matsui et al. (2017)
Gracixalus quangi	Nghe An, Vietnam	AMS R173417	JN862539	Rowley et al. (2011)
Gracixalus supercornutus	Kon Tum, Vietnam	AMS R173887	JN862545	Rowley et al. (2011)
Gracixalus lumarius	Kon Tum, Vietnam	AMS R 176202	KF918412	Rowley et al. (2014)
Gracixalus jinggangensis	Mt. Jinggang, Jiangxi, China	SYS a003186	KY624587	Zeng et al. (2017)
Gracixalus seesom	Kanchanaburi, Thailand	KUHE 35084	LC011932	Matsui et al. (2015)
Gracixalus quyeti	Cha Noi, Vietnam	VNUH 160706	EU871428	Nguyen et al. (2008)
Gracixalus sp.	Wenshan, Yunnan, China	03320Rao	GQ285669	Li et al. (2009)
Gracixalus gracilipes	Lao Cai, Vietnam	AMS R 177672	KT374014	Rowley et al. (2015)
Gracixalus gracilipes	Pingbian, Yunnan, China	060821196Rao	GQ285668	Li et al. (2009)
Gracixalus ziegleri	Yen Bai, Vietnam	MCC.2018.35	LC642813	Le et al. (2021)
Gracixalus ziegleri	Yen Bai, Vietnam	MCC.2018.15	LC642812	Le et al. (2021)
Gracixalus trieng	Kon Tum Province, Vietnam	AMS R176206	MT328246	Rowley et al. (2020)
Gracixalus trieng	Kon Tum Province, Vietnam	UNS 00342/AMS R176205	MT328245	Rowley et al. (2020)
Gracixalus tianlinensis	Guangxi, China	NHMG 1705016	MH117961	Chen et al. (2018)
Gracixalus tianlinensis	Guangxi, China	NHMG 1705015	MH117960	Chen et al. (2018)
Gracixalus guangdongensis	Hunan, China	CIB HN201108200	LC011936	Matsui et al. (2015)
Gracixalus guangdongensis	Guangdong, China	SYS a004902	MG520193	Wang et al. (2018)
Gracixalus guangdongensis	Guangdong, China	SYS a005750	MG520197	Wang et al. (2018)
Gracixalus medogensis	Medog, Xizang, China	KIZ010956	MW023606	Xu et al. (2020)
Gracixalus huaping	Huaping, Guangxi, China	GXNU YU000480	PP083976	Luo et al. (2025)
Gracixalus huaping	Huaping, Guangxi, China	GXNU YU000511	PP083977	Luo et al. (2025)
Gracixalus huaping	Huaping, Guangxi, China	GXNU YU000512	PP083978	Luo et al. (2025)
Gracixalus huaping	Huaping, Guangxi, China	GXNU YU000513	PP083979	Luo et al. (2025)
Gracixalus huaping	Huaping, Guangxi, China	GXNU YU000514	PP083980	Luo et al. (2025)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	KIZ 060821013	EF564524	Yu et al. (2008)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	KIZ 061210YP	EU215525	Li et al. (2008)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	SYS a002183	KY624585	Zeng et al. (2017)
Gracixalus jinxiuensis	Jinxiu, Guangxi, China	SYS a002182	KY624584	Zeng et al. (2017)
Gracixalus truongi	Tuan Giao District, Dien Bien Province, Vietnam	IEBR A.5006	OP750514	Tran et al. (2023)
Gracixalus truongi	Tuan Giao District, Dien Bien Province, Vietnam	IEBR A.5005	OP750513	Tran et al. (2023)
Gracixalus nonggangensis	Guangxi, China	NHMG111024	JX841317	Mo et al. (2013)
Gracixalus nonggangensis	Guangxi, China	NHMG20091009	JX841319	Mo et al. (2013)
Gracixalus nonggangensis	Guangxi, China	NHMG200910010	JX841320	Mo et al. (2013)
Gracixalus ananjevae	Nghe An, Vietnam	VNMN 03012	JN862546	Rowley et al. (2011)
Gracixalus patkaiensis	Northeast India	WII-ADA1352	OQ940026	Boruah et al. (2023)
Gracixalus patkaiensis	Northeast India	WII-ADA1353	OQ940027	Boruah et al. (2023)
Gracixalus weii	Leigongshan, Guizhou, China	MT LS20240517001	PP852208	Liu et al. (2025)
Gracixalus weii	Leigongshan, Guizhou, China	MT LS20240517002	PP852209	Liu et al. (2025)
Gracixalus weii	Leigongshan, Guizhou, China	MT LS20240518009	PP852212	Liu et al. (2025)
Gracixalus weii	Leigongshan, Guizhou, China	MT LS20240518010	PP852213	Liu et al. (2025)
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000795	PV746479	This study
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000796	PV746480	This study
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000962	PV746481	This study
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000978	PV746482	This study
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000979	PV746483	This study
Gracixalus liusanjieae **sp. nov.**	Mao'er Mountain, Guangxi, China	GXNU YU000980	PV746484	This study

We selected *Rhacophorus borneensis* Matsui, Shimada & Sudin,2013 and *Kurixalus idiootocus* (Kuramoto & Wang, 1987) as outgroup according to Luo et al. (2025) and sequences of them were also downloaded from GenBank.

Sequences were aligned in MEGA v.11 (Tamura et al. 2021) using MUSCLE with the default parameters. Alignments were visually checked by eye for accuracy and trimmed to minimize missing characters. Uncorrected pairwise distances were calculated in MEGA v. 11. The best substitution model (GTR + I + G) was selected using the corrected Akaike Information Criterion (**AICc**) in jMODELTEST v. 2.1.10 (Darriba et al. 2012), and then we used maximum likelihood and Bayesian inference to construct the phylogeny of the genus *Gracixalus*. The maximum likelihood analysis was performed in RaxmlGUI v. 2.0 (Edler et al. 2021) with 1000 rapid bootstrap replicates.

Bayesian inference was conducted in MrBayes v. 3.2.6 (Ronquist et al. 2012) based on the selected substitution model. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 3000,000 generations and sampled every 100 generations. Convergence and burn-in were checked using the program Tracer v. 1.6. (Rambaut et al. 2014). The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs).

We conducted analysis of species delimitation using the method of Assemble Species by Automatic Partitioning (**ASAP**; Puillandre et al. 2020) and the model of simple distance (p-distance). The partitioning with the lowest ASAP score was chosen as the best according to Puillandre et al. (2020).

Morphological analysis

Morphometric data were taken using electronic digital calipers to the nearest 0.1 mm. The morphological terminologies followed Yu et al. (2019). A total of 13 characters were measured, including snout-vent length (**SVL**), head length (**HL**), head width (**HW**), snout length (**SL**), internarial distance (**IND**), interorbital distance (**IOD**), eye diameter (**ED**), upper eyelid width (**UEW**), tympanum diameter (**TD**), forearm and hand length (**FHL**), tibia length (**TL**), length of tarsus and foot (**TFL**), and foot length (**FL**).The webbing formula followed Myers and Duellman (1982).

We used the t-test in SPSS v. 17.0 to evaluate the differences in quantitative characters of adult males between the new species and its closest relatives revealed by phylogenetic analyses. Principal component analyses (PCA) were conducted based on a correlation matrix of size-corrected measurements of males in SPSS. Scatterplots of the first two PCA factors were used to present the morphological differentiation.

Bioacoustics analysis

Advertisement calls were recorded using SONY PCM-A10 digital sound recorder in the field. Calls of an individual of the new species were recorded on 26 April 2025 at ambient temperature of 15 °C, calls from an individual of *G. jinxiuensis* were recorded in Jinxiu, Guangxi, China on 10 June 2024 at ambient temperature of 22 °C, and calls of an individual of *G. huaping* were recorded in Huaping, Guangxi, China on 4 May 2022 at ambient temperature of 20 °C. The sound files were sampled at 44.1 kHz with 16 bits in depth. Number of notes, call duration, interval of calls, frequency range, and dominant frequency were measured using Raven Pro 1.6 (www.birds.cornell.edu/raven). The bioacoustics data of *G. guangdongensis* Wang, Zeng, Liu & Wang, 2018, *G. jinggangensis* Zeng, Zhao, Chen, Chen, Zhang & Wang, 2017, *G. nonggangensis*, and *G. tianlinensis* were obtained from Wang et al. (2018), Zeng et al. (2017), and Chen et al. (2018), respectively, for comparison.

Results

Phylogenetic relationship and species delineation

After cutting off both ragged sides, length of the obtained alignment of 16S rRNA sequence is 547 bp. Both maximum likelihood analysis and Bayesian inference recovered the samples of *Gracixalus* from Mao'er Mountain, Guangxi, China as a distinct lineage. This lineage is closely related to *G. weii* Liu, Peng, Wang, Feng, Shen, Li, Chen, Su & Tang, 2025 and the clade of *G. jinxiuensis* and *G. huaping* with strong support, although the relationships between them were not resolved well (Fig. 2). The genetic distances (uncorrected p-distance) between this novel linage and its congeners ranged from 1.8% (vs. *G. weii*) to 13.5% (vs. *G. lumarius* Rowley, Le, Dau, Hoang & Cao, 2014) (Suppl. material 1).

The best partition (score = 4.0) obtained by the ASAP analysis placed the samples of *Gracixalus* included in this study into 23 operational taxonomic units (OUT), one of which corresponds to the lineage consisted of samples from Mao'er Mountain and other 22 OUT correspond to 21 known species and an unnamed lineage from Yunnan (Fig. 3).

Morphology

Morphometric data are summarized in Table 2. The t-tests revealed that the male specimens from Mao'er Mountain differ significantly ($p < 0.05$) from males of *G. huaping* in snout length (SL), internarial distance (IND), and tibia length (TL; Table 3); from males of *G. jinxiuensis* in forearm and hand length (FHL) and foot length (FL; Table 4); and from males of *G. weii* in head length (HL), snout length (SL), internarial distance (IND), upper eyelid width (UEW), eye diameter (ED), tympanum diameter (TD), tibia length (TL), length of tarsus and foot (TFL), and foot length (FL; Table 5).

PCA analysis illustrated that the first two principal components accounted for 62.30% of the total variance (Table 6). Loadings for PC1 accounted for 40.65% of the total variance and were most heavily loaded on IND and TFL, which can separate the Mao'er Mountain population from *G. jinxiuensis*, *G. huaping*, and *G. weii* (Fig. 4). Loadings for PC2 accounted for 21.65% and were heavily loaded on SL and FL, which showed that generally the Mao'er Mountain population can be separated from *G. weii* and *G. jinxiuensis* by having shorter snout and longer foot (Table 6, Fig. 4).

Additionally, in terms of advertisement calls, the Mao'er Mountain population can be distinguished from other species by number of notes, call duration, or absence of harmonics (see below). Therefore, based on the

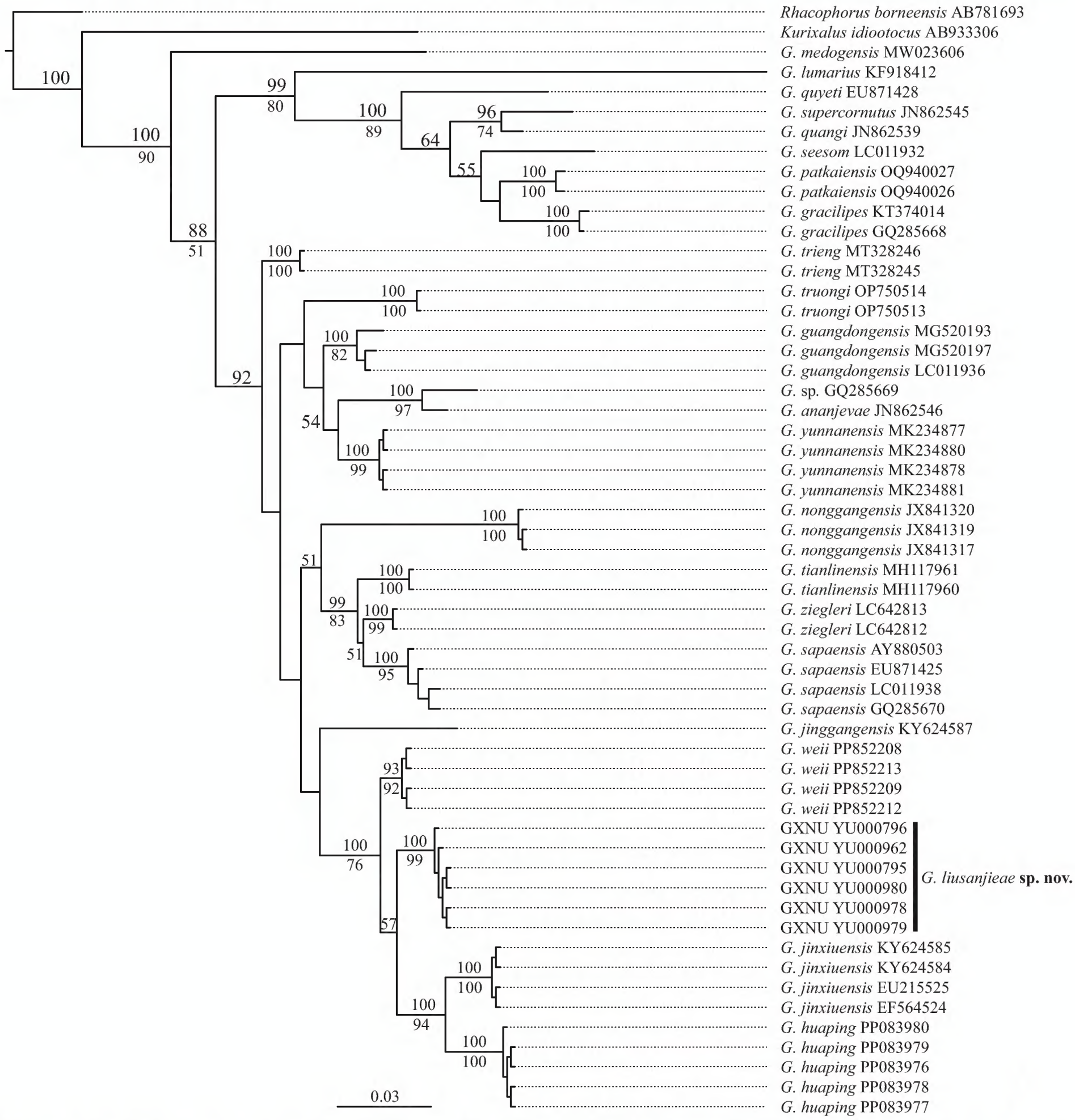

Figure 2. Bayesian phylogram of *Gracixalus* inferred from 547 bp of 16S rRNA gene. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values above 50% are shown), respectively.

integrative evidence of morphological, molecular, and bioacoustics analyses, we consider that the samples of *Gracixalus* from Mao'er Mountain, Guangxi, China represent a new species and describe it herein.

Taxonomic account

Gracixalus liusanjieae sp. nov.

https://zoobank.org/6F021E10-7516-4719-A7AF-3A67D15F2254

Figs 5–8

Type material. ***Holotype.*** • GXNU YU000978, an adult male collected on 4 April 2025 by Guohua Yu, Yuanqiang Pan, and Xiangjian Wu from Maoershan National Natural Reserve, Guilin, Guangxi, China (25.911°N, 109.466°E, 1573 m elevation).

Paratypes. • Two adult males (GXNU YU000795, GXNU YU000796) collected on 26 April 2024 by Guohua Yu, Yuanqiang Pan, and Xiangjian Wu, • an adult male (GXNU YU000962) collected on 13 August 2024 by Tongxiang Zou, and • two adult females (GXNU YU000979 and GXNU YU000980) collected on 26 April 2025 by Guohua Yu, Yuanqiang Pan, and Xiangjian Wu. All paratypes were collected from the type locality.

Etymology. The specific epithet is named for Sanjie Liu, referring to a famous woman in Chinese ancient legend who came from Guangxi and who was known for her

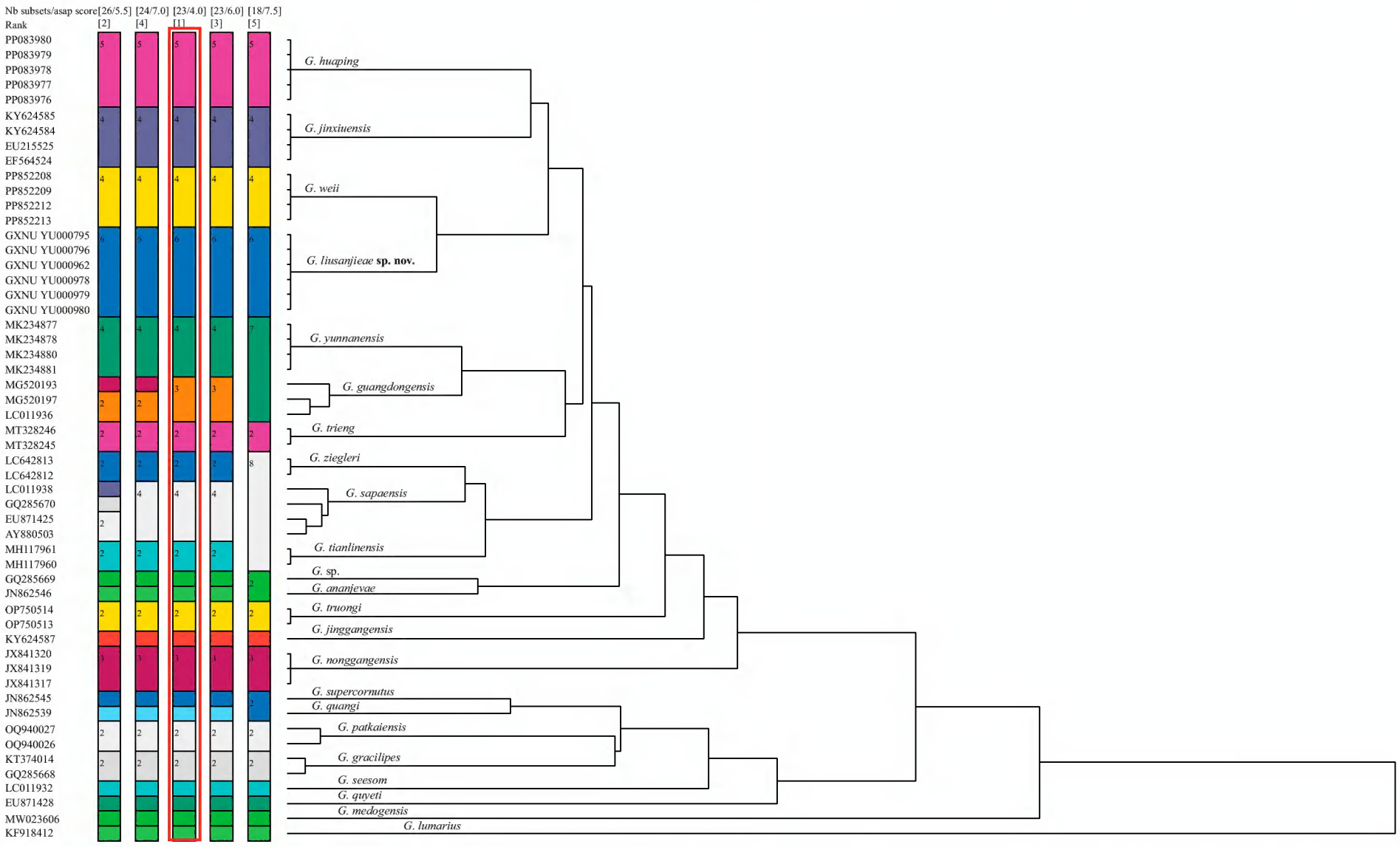

Figure 3. ASAP species delimitation in genus *Gracixalus* based on 16S sequences. The best partition with the lowest score is highlighted with a red frame.

Table 2. Measurements (mm) of *Gracixalus liusanjieae* sp. nov., *G. jinxiuensis*, *G. huaping*, and *G. weii*. The data of *G. weii* was obtained from Liu et al. (2025).

Character	*Gracixalus liusanjieae* sp. nov.		*G. jinxiuensis*	*G. huaping*		*G. weii*	
	Male (n = 4)	Female (n = 2)	Male (n = 4)	Male (n = 3)	Female (n = 4)	Male (n = 5)	Female (n = 2)
SVL	27.5–33.2 (30.8 ± 2.4)	38.1–39.7	27.4–30.8 (29.6 ± 1.5)	26.6–28.8 (28.0 ± 1.2)	29.8–32.8 (31.5 ± 1.3)	30.1–34.0 (31.9 ± 1.4)	35.2–36.0
HL	9.8–11.4 (10.5 ± 0.7)	13.1–13.6	9.9–11.2 (10.4 ± 0.6	9.3–9.9 (9.7 ± 0.3)	10.5–11.7 (11.0 ± 0.5)	9.5–10.8 (10.3 ± 0.5)	11.3–11.8
HW	10.2–12.1 (11.5 ± 0.9)	13.6–14.0	10.7–11.5 (11.0 ± 0.4)	10.3–10.9 (10.7 ± 0.3)	11.0–12.4 (11.7 ± 0.6)	11.6–12.8 (12.1 ± 0.4)	12.4–13.1
SL	3.6–4.2 (4.1 ± 0.3)	5.1–5.4	4.0–5.0 (4.6 ± 0.5)	3.0–3.2 (3.1 ± 0.1)	3.5–3.8 (3.7 ± 0.1)	4.7–5.1 (4.9 ± 0.2)	5.2–5.4
IND	3.1–3.6 (3.3 ± 0.2)	3.6–3.9	2.6–3.5 (3.1 ± 0.4)	3.0–3.4 (3.2 ± 0.2)	3.2–3.6 (3.4 ± 0.2)	3.8–4.5 (4.1 ± 0.3)	4.0–4.8
IOD	3.2–3.7 (3.5 ± 0.2)	4.4–4.5	3.5–4.3 (3.7 ± 0.4)	3.2–3.4 (3.3 ± 0.1)	3.4–3.8 (3.6 ± 0.2)	3.4–4.2 (3.7 ± 0.4)	3.7–4.4
UEW	2.3–3.3 (2.9 ± 0.4)	3.3–3.4	2.6–2.9 (2.7 ± 0.1)	2.5–2.7 (2.6 ± 0.1)	2.6–3.2 (2.9 ± 0.3)	2.3–2.9 (2.5 ± 0.2)	2.7–3.0
ED	3.8–4.0 (3.9 ± 0.1)	4.2–4.3	3.7–4.1 (3.9 ± 0.2)	3.2–3.3 (3.2 ± 0.1)	3.3–3.6 (3.5 ± 0.1)	3.3–3.7 (3.6 ± 0.2)	4.4–4.9
TD	1.5–1.7 (1.6 ± 0.1)	2.0–2.2	1.5–2.0 (1.7 ± 0.2)	1.5–1.6 (1.5 ± 0.1)	1.7–1.9 (1.8 ± 0.1)	2.2–2.6 (2.4 ± 0.2)	2.7–3.4
FHL	13.9–17.4 (15.7 ± 1.4)	18.4–19.6	13.4–14.7 (14.2 ± 0.6)	13.6–14.7 (14.2 ± 0.6)	15.7–16.4 (16.0 ± 0.3)	15.9–17.6 (16.7 ± 0.7)	18.0–18.2
TL	13.3–15.8 (14.7 ± 1.1)	18.2–18.5	13.8–14.7 (14.1 ± 0.4)	13.2–14.7 (14.0 ± 0.8)	14.9–15.7 (15.3 ± 0.4)	14.9–17.2 (15.8 ± 0.9)	17.3–17.4
TFL	18.2–22.8 (21.0 ± 2.0)	25.4–26.6	18.2–20.5 (19.2 ± 1.0)	18.6–20.4 (19.5 ± 0.9)	21.8–22.4 (22.0 ± 0.3)	21.9–23.7 (22.6 ± 1.2)	24.5–24.6
FL	12.1–15.5 (14.0 ± 1.4)	17.3–17.4	11.5–13.4 (12.4 ± 0.8)	11.8–13.0 (12.4 ± 0.6)	14.0–14.5 (14.2 ± 0.2)	12.1–14.8 (13.6 ± 1.0)	14.7–15.2

exceptional singing talent. The specific name means that the new species is distributed in Guangxi and its advertisement calls are melodic. We suggested "Maoershan small tree frog" for the common English name and "刘三姐纤树蛙 (Liú Sān Jiě Xiān Shù Wā)" for the common Chinese name.

Diagnosis. The new species is assigned to genus *Gracixalus* based upon the following set of morphological characters: tips of digits enlarged to discs bearing circum-marginal grooves, vomerine teeth absent, inner (first and second) and outer (third and fourth) fingers not opposable, and an inversed Y-shaped dark brown marking on the dorsum (Fei 1999; Yu et al. 2019; Tran et al. 2023).

The new species can be distinguished from its congeners by a combination of the following morphological characters: (1) SVL 27.5–33.2 mm in males and 38.1–39.7 mm in females; (2) dorsal surface beige to brown; (3) dorsal surface rough with a few flatten tubercles on dorsum; (4) internal vocal sac in males, vocal sac opening slitted; (5) throat relatively smooth with barely visible tubercles; (6) finger webbing rudimentary; (7) linea masculina absent; (8) snout rounded; (9) tibiotarsal articulation reaching center of eye; (10) tibiotarsal projection absent; (11) nuptial pads present on fingers I and II; (12) heels slightly overlapping when legs at right angle to body; (13) ventral surface translucent, creamy white with dark blotches; (14) belly granular.

Description of holotype. Small size (SVL 33.2 mm); head wider (HW 12.1 mm) than long (HL 11.4 mm); snout rounded, slightly projecting beyond margin of lower jaw in ventral view, rounded in profile; canthus rostralis rounded; nostril oval, protuberant, closer to tip of snout than eye; loreal region oblique, slightly concave;

Table 3. Results of t-test between males of *G. liusanjieae* sp. nov. and *G. huaping* based on the size-corrected data except SVL (*p < 0.05).

Character	Mean ± SD (n = 4)	Mean ± SD (n = 3)	Levene's test		t-test	
	G. liusanjieae sp. nov.	*G. huaping*	F	p-value	t	p-value
SVL	30.8 ± 2.4	28.0 ± 1.2	0.699	0.441	1.841	0.125
HL	0.342 ± 0.011	0.345 ± 0.005	1.485	0.277	-0.501	0.638
HW	0.372 ± 0.010	0.381 ± 0.006	0.681	0.447	-1.448	0.207
SL	0.131 ± 0.004	0.112 ± 0.001	3.489	0.121	8.754	0.000*
IND	0.107 ± 0.004	0.117 ± 0.001	4.290	0.093	-3.570	0.016*
IOD	0.112 ± 0.011	0.118 ± 0.002	2.483	0.176	-0.872	0.423
UEW	0.094 ± 0.008	0.092 ± 0.004	1.415	0.288	0.398	0.707
ED	0.127 ± 0.010	0.116 ± 0.004	0.667	0.451	1.872	0.120
TD	0.052 ± 0.003	0.055 ± 0.002	0.541	0.495	-1.390	0.223
FHL	0.509 ± 0.014	0.507 ± 0.006	1.749	0.243	0.208	0.844
TL	0.476 ± 0.007	0.501 ± 0.008	0.128	0.735	-4.214	0.008*
TFL	0.681 ± 0.126	0.695 ± 0.015	0.150	0.715	-1.397	0.221
FL	0.452 ± 0.011	0.442 ± 0.009	0.201	0.673	1.142	0.305

Table 4. Results of t-test between males of *G. liusanjieae* sp. nov. and *G. jinxiuensis* based on the size-corrected data except SVL (*p < 0.05).

Character	Mean ± SD (n = 4)	Mean ± SD (n = 4)	Levene's test		t-test	
	G. liusanjieae sp. nov.	*G. jinxiuensis*	F	p-value	t	p-value
SVL	30.8 ± 2.4	29.6 ± 1.5	0.445	0.529	0.846	0.430
HL	0.342 ± 0.011	0.351 ± 0.013	1.112	0.332	-1.092	0.317
HW	0.372 ± 0.010	0.371 ± 0.015	0.763	0.416	0.077	0.941
SL	0.131 ± 0.004	0.154 ± 0.021	21.325	0.004	-2.096	0.122
IND	0.107 ± 0.004	0.103 ± 0.008	0.661	0.447	0.994	0.359
IOD	0.112 ± 0.011	0.126 ± 0.013	0.767	0.415	-1.603	0.160
UEW	0.094 ± 0.008	0.092 ± 0.003	3.742	0.101	0.408	0.697
ED	0.127 ± 0.010	0.133 ± 0.008	0.100	0.763	-0.904	0.401
TD	0.052 ± 0.003	0.058 ± 0.006	0.803	0.405	-1.804	0.121
FHL	0.509 ± 0.014	0.478 ± 0.011	0.098	0.765	3.582	0.012*
TL	0.476 ± 0.007	0.477 ± 0.020	1.763	0.232	-0.029	0.978
TFL	0.681 ± 0.126	0.648 ± 0.024	1.114	0.332	2.365	0.056
FL	0.452 ± 0.011	0.418 ± 0.014	0.096	0.767	3.877	0.008*

Table 5. Results of t-test between males of *G. liusanjieae* sp. nov. and *G. weii* based on the size-corrected data except SVL (*p < 0.05).

Character	Mean ± SD (n = 4)	Mean ± SD (n = 5)	Levene's test		t-test	
	G. liusanjieae sp. nov.	*G. weii*	F	p-value	t	p-value
SVL	30.8 ± 2.4	31.9 ± 1.4	0.871	0.382	0.867	0.415
HL	0.342 ± 0.011	0.324 ± 0.009	0.001	0.982	-2.678	0.032*
HW	0.372 ± 0.010	0.380 ± 0.013	0.139	0.720	1.052	0.328
SL	0.131 ± 0.004	0.153 ± 0.003	0.014	0.909	9.223	0.000*
IND	0.107 ± 0.004	0.128 ± 0.005	0.194	0.673	6.404	0.000*
IOD	0.112 ± 0.011	0.117 ± 0.009	0.053	0.824	0.771	0.466
UEW	0.094 ± 0.008	0.079 ± 0.008	0.015	0.906	-2.758	0.028*
ED	0.127 ± 0.010	0.112 ± 0.006	0.150	0.710	-2.863	0.024*
TD	0.052 ± 0.003	0.074 ± 0.004	0.072	0.796	10.020	0.000*
FHL	0.509 ± 0.014	0.522 ± 0.018	0.773	0.409	1.182	0.276
TL	0.476 ± 0.007	0.495 ± 0.011	0.417	0.539	2.841	0.025*
TFL	0.681 ± 0.126	0.709 ± 0.015	1.638	0.242	2.957	0.021*
FL	0.452 ± 0.011	0.426 ± 0.018	1.716	0.232	-2.487	0.042*

interorbital distance (IOD 3.7 mm) nearly equal to internarial distance (IND 3.6 mm) and upper eyelid width (UEW 3.3 mm); eye large, horizontal diameter

Table 6. Factor loadings of first two principal components of 12 size-adjusted morphometric characteristics of *Gracixalus liusanjieae* sp. nov., *G. jinxiuensis*, *G. huaping*, and *G. weii*.

Character	PC1	PC2
Variance	4.878	2.597
% of var.	40.653	21.645
Cumulative % of var.	40.653	62.298
HL	–0.728	–0.125
HW	0.453	–0.011
SL	0.089	0.839
IND	0.908	0.023
IOD	–0.208	0.664
UEW	–0.645	–0.437
ED	–0.734	0.298
TD	0.768	0.515
FHL	0.769	–0.192
TL	0.632	–0.088
TFL	0.856	–0.376
FL	–0.027	–0.840

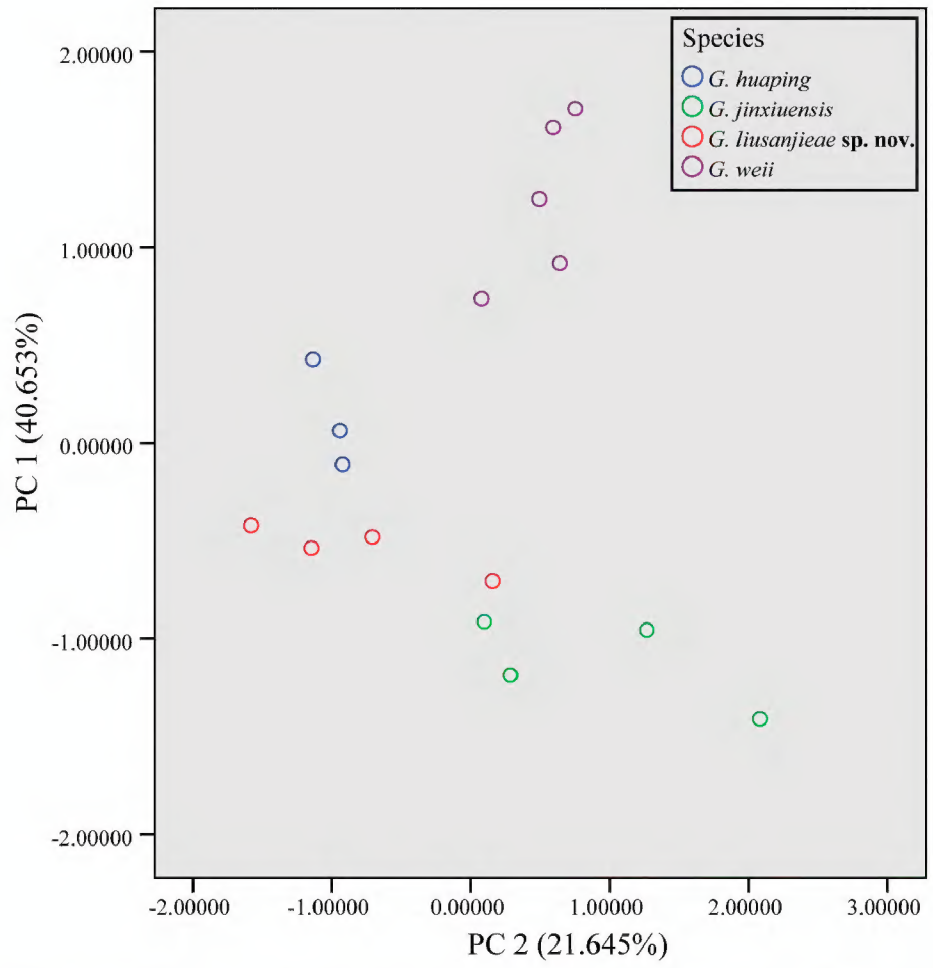

Figure 4. Scatterplot of principal components 1 and 2 of size-adjusted morphometric data of *Gracixalus liusanjieae* sp. nov., *G. huaping*, *G. jinxiuensis*, and *G. weii*.

(ED 3.8 mm) slightly shorter than snout length (SL 4.2 mm); pupil oval, horizontal; tympanum distinct (TD 1.6 mm), slightly smaller than half of ED; supratympanic fold distinct, extending from posterior corner of eye to above insertion of arm; vomerine teeth absent; tongue notched posteriorly; single internal vocal sac, a pair of vocal sac slits on floor of mouth at both corners.

Forelimbs relatively strong; forearm and hand length (FHL 17.4 mm) longer than 50% of SVL; relative finger lengths I < II < IV < III; finger webbing rudimentary; tips of all fingers expanded into discs with circum-marginal grooves; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2, the proximal one smaller than the distal one on fingers III and IV; two metacarpal tubercles, the outer divided into two; nuptial pads white, present on the base of the first and the second fingers.

Figure 5. Views of the holotype of *Gracixalus liusanjieae* sp. nov. (voucher no. GXNU YU000978) in life. Nuptial pads were highlighted with arrows (Photo by Guohua Yu).

Figure 6. Dorsal and ventral views of the holotype of *Gracixalus liusanjieae* sp. nov. (voucher no. GXNU YU000978) in preservative (Photo by Xiangjian Wu).

Heels meeting when legs at right angle to body; the relative lengths of the toes I < II < III < V < IV; tibiotarsal articulation reaching the middle of the eye when hindlimb adpressed to body; tip of toes expanded into discs with circum-marginal grooves; toe discs slightly smaller than finger discs; half web between toes, webbing formula I2–2⅓II1½–3III1½–2⅔IV2⅓–1⅔V; subarticular tubercles prominent, round, formula 1, 1, 2, 3, 2; a few supernumerary tubercles present; inner metatarsal tubercle oval, outer metatarsal tubercle absent.

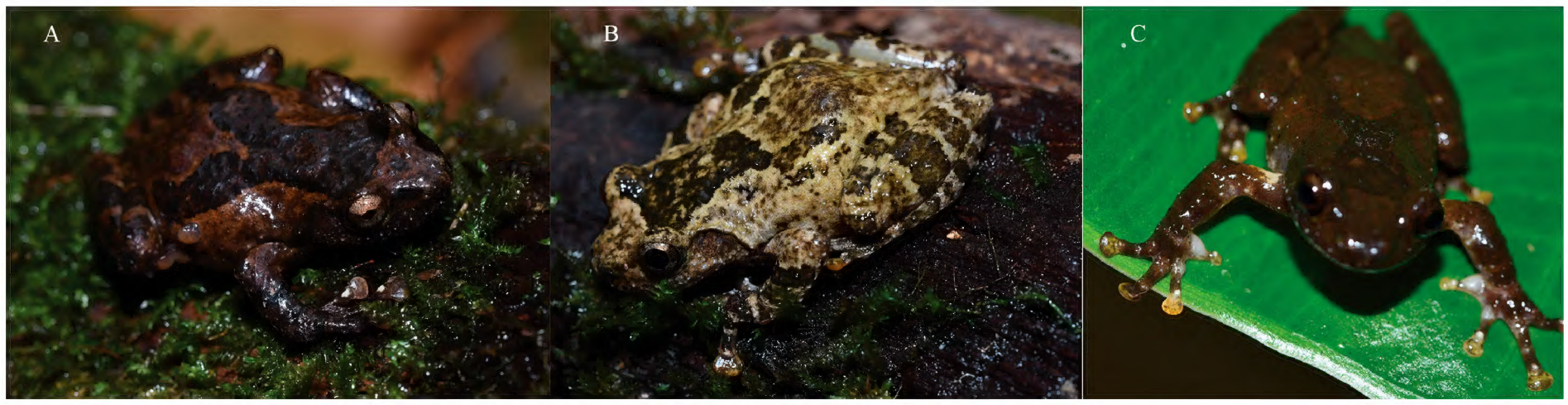

Figure 7. Paratypes GXNU YU000979 (**A**), GXNU YU000980 (**B**), and GXNU YU000795 (**C**) in life (**A, B.** Photo by Yuanqiang Pan; **C.** Photo by Guohua Yu).

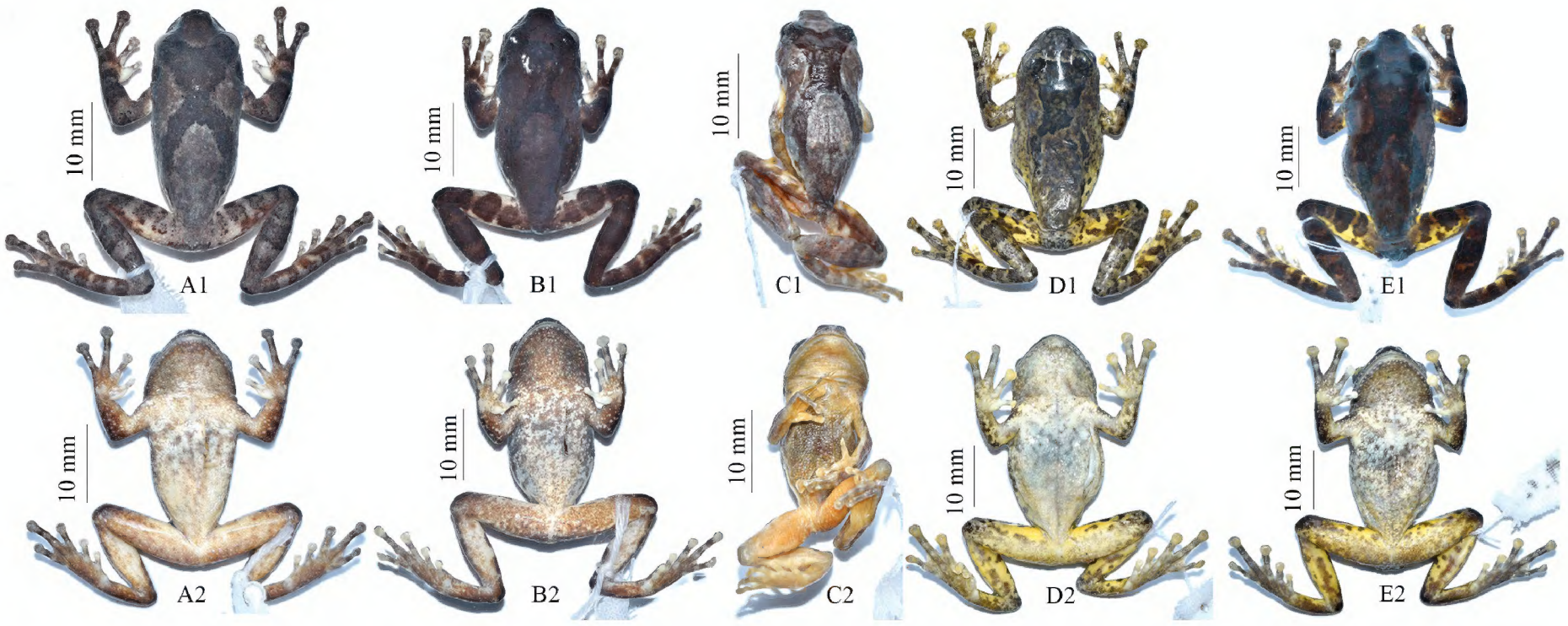

Figure 8. Dorsal and ventral surfaces of paratypes GXNU YU000796 (**A**), GXNU YU000795 (**B**), GXNU YU000962 (**C**), GXNU YU000980 (**D**), and GXNU YU000979 (**E**) in preservative (Photo by Xiangjian Wu).

Dorsal surface rough, sparsely scattered with a few small flatten tubercles on the upper eyelids, head, dorsum, and limbs; white tubercles scattered on temporal region, edge of upper and lower jaw, and dorsal surface of limbs; flank rough, scattered with white tubercles; throat relatively smooth with barely visible tubercles; chest and belly granular, and ventral surface of thighs rough scattered with white tubercles.

Coloration of holotype. In life, dorsal surface beige; a dark brown inverted Y-shaped marking on dorsal surface covering interorbital region, bifurcating into two branches on shoulder and extending posteriorly; dorsal surface of limbs beige with dark brown stripes; anterior and posterior parts of thigh light yellow; ventral surface of thigh light yellow mottled with light purple; discs of fingers and toes light yellow; flanks beige; throat and chest creamy white with dark speckles; iris bronze (Fig. 5).

In preservative, color faded but pattern same as in life; dorsal surface gray-brown with a dark brown inverted Y-shaped marking; dark brown stripes on limbs; ventral surface dirty white with dark speckles (Fig. 6).

Sexual dimorphism. Females are larger than males in body size. Males have white nuptial pads on the base of fingers I & II (Figs 5, 7C) and internal single subgular vocal sac.

Morphological variation. The dorsal surface of paratypes GXNU YU000795 and GXNU YU000979 is dark brown, whereas the dorsal surface of other types is beige or brown (Figs 7, 8). Paratypes GXNU YU000962 and GXNU YU000980 have less dark blotches on ventral surface (Fig. 8). In addition, the inverted Y-shaped marking on the dorsum in paratype GXNU YU000980 is discontinuous (Fig. 8).

Advertisement call. The advertisement calls of the new species consist of two notes (Fig. 9A). The call duration of the new species is 0.66–0.80 s (0.74 ± 0.04; Table 7) and the peak frequency is 2.58 kHz. The frequency range is 2.2–2.9 kHz and the interval between calls ranges from 5.6 s to 18.2 s (8.9 ± 2.9).

Distribution and ecology. Currently, the new species is known only from the type locality. The new species inhabits bamboo forest (Fig. 10) and enters breeding season in early April. Eggs with gel nests were found on the broken branches placed on the bottles that were used as artificial breeding trap for surveillance of tree frogs (Fig. 10), and calls were heard during the surveys. *Zhangixalus minimus* (Rao, Wilkinson & Liu, 2006) was also found at the type locality.

Measurements of holotype (in mm). SVL 33.2, HL 11.4, HW 12.1, SL 4.2, IND 3.6, IND 3.7, UEW 3.3, ED 3.8, TD 1.6, FHL 17.4, TL 15.8, TFL 22.8, FL 15.5.

Comparisons. Phylogenetically, the new species is closer to *G. jinxiuensis*, *G. huaping*, and *G. weii* than to other species. *Gracixalus liusanjieae* sp. nov. is distinguishable from *G. jinxiuensis*, with which the new species has previously been confused, by nuptial pads distinct, white, present on the base of fingers I and II

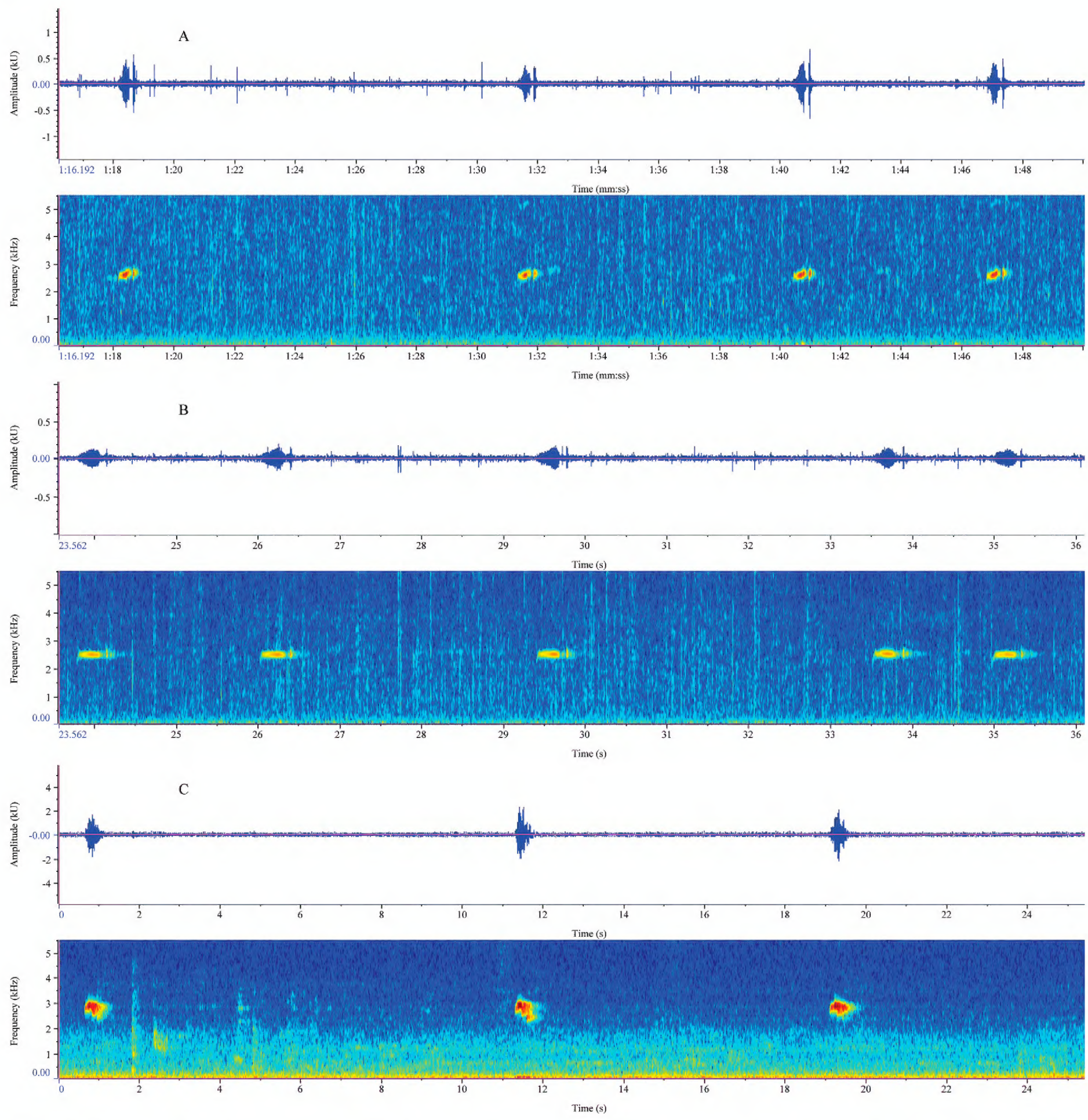

Figure 9. Waveforms and spectrograms of the advertisement calls of *Gracixalus liusanjieae* sp. nov. (**A**), *G. jinxiuensis* (**B**), and *G. huaping* (**C**).

Table 7. The measurements of the advertisement calls of *G. liusanjieae* sp. nov., *G. jinxiuensis*, and *G. huaping*.

Species	Number of calls	Number of notes	Call duration (s)	Call interval (s)	Peak frequency (kHz)	Frequency range (kHz)
G. liusanjieae **sp. nov.**	20	2	0.66–0.80 (0.74 ± 0.04)	5.6–18.2 (8.9 ± 2.9)	2.58	2.25–2.95
G. jinxiuensis	32	2	0.46–0.61 (0.51 ± 0.03)	0.99–14.43 (3.78 ± 2.66)	2.50	2.08–2.95
G. huaping	3	1	0.61–0.69 (0.64 ± 0.04)	7.18–10.03	2.89	2.10–3.37

(vs. nuptial pads not distinct, light yellow, present on the base of finger I), longer forearm and hand (FHL/SVL 0.509 ± 0.0136 vs. 0.4779 ± 0.0107), longer foot (FL/SVL0.452 ± 0.0113 vs. 0.417 ± 0.0135), and longer duration of advertisement calls (0.74 ± 0.04 vs. 0.51 ± 0.03; Table 7); from *G. weii* by nuptial pads present on fingers I and II (vs. on finger I), larger body size in females (SVL 38.1–39.7 mm vs. 35.2–36.0 mm), longer head (HL/SVL 0.342 ± 0.011 vs. 0.324 ± 0.009), shorter snout (SL/SVL 0.131 ± 0.0037 vs. 0.153 ± 0.0034), narrower internarial distance (IND/SVL 0.107 ± 0.004 vs. 0.128 ± 0.005), shorter tibia (TL/SVL 0.476 ± 0.007 vs. 0.4945 ± 0.011), shorter tarsus and foot (TFL/SVL 0.681 ± 0.123 vs. 0.709 ± 0.015), and longer foot (FL/SVL 0.452 ± 0.011 vs. 0.426 ± 0.018); and from *G. huaping* by bigger body size (SVL 27.5–33.2 mm in males and 38.1–39.7 mm in females vs. SVL 26.6–28.8 mm in males and 29.8–32.8 mm in females), linea masculina

Figure 10. Habitat of *Gracixalus liusanjieae* sp. nov. at the type locality (**A**) and eggs of the new species laid on broken branches (**C**) that was placed on an artificial breeding trap (**B**) (**A, B.** Photo by Guohua Yu; **C.** Photo by Yuanqiang Pan).

absent (vs. present), vocal sac opening slitted (vs. oval and rim of the opening dark brown), nuptial pads present on fingers I and II (vs. on finger I), and advertisement calls composed of two notes (vs. one note; Fig. 9).

The new species can be distinguished from *G. ananjevae* (Matsui & Orlov, 2004) by nuptial pads present on fingers I and II (vs. on finger I), finger webbing rudimentary (vs. finger webbing formula I2–2II2–basalIII3–2.75IV) and the presence of white granules around anus and on limbs (vs. absent); from *G. carinensis* (Boulenger, 1893) by smaller body size (SVL 27.5–31.5 mm in males and 38.1–39.7 mm in females vs. 30.2–38.1 mm in males and 40.3–41.8 mm in females), less developed toe webbing (Fig. 11), and tubercles on dorsum present (vs. absent); from *G. gracilipes* by bigger body size (SVL 27.5–31.5 mm in males and 38.1–39.7 mm in females vs. 20–24 mm in males and ca. 30 mm in females), snout rounded (vs. triangularly pointed), and dorsal surface beige to brown (vs. greenish); from *G. guangdongensis* by nuptial pads present on fingers I and II (vs. on finger I), bigger body size in females (38.1–39.7 mm vs. 34.9–35.4 mm), and longer duration of advertisement calls (0.74 ± 0.04 vs. 0.56 ± 0.07); from *G. jinggangensis* by heels overlapping (vs. just meeting) and calls composed of two notes with no harmonics (vs. three or four notes with multiple harmonics); from *G. lumarius* by smaller body size (SVL 27.5–31.5 mm in males vs. 38.9–41.6 mm in males), dorsal surface rough sparsely scattered with flatten tubercles (vs. dorsal surface of head and back covered in dense and distinctive white conical tubercles), nuptial pads present on fingers I and II (vs. on finger I), and supratympanic fold distinct (vs. indistinct); and from *G. medogensis* (Ye & Hu, 1984) by dark brown mark beginning between eyes bifurcated into two wide branches after the back of shoulders (vs. bifurcated into two relatively narrow branches before the back of shoulders), dorsal color beige to brown (vs. grass green), finger webbing rudimentary (vs. absent), linea masculine absent (vs. present), and nuptial pads present on fingers I and II (vs. on finger I).

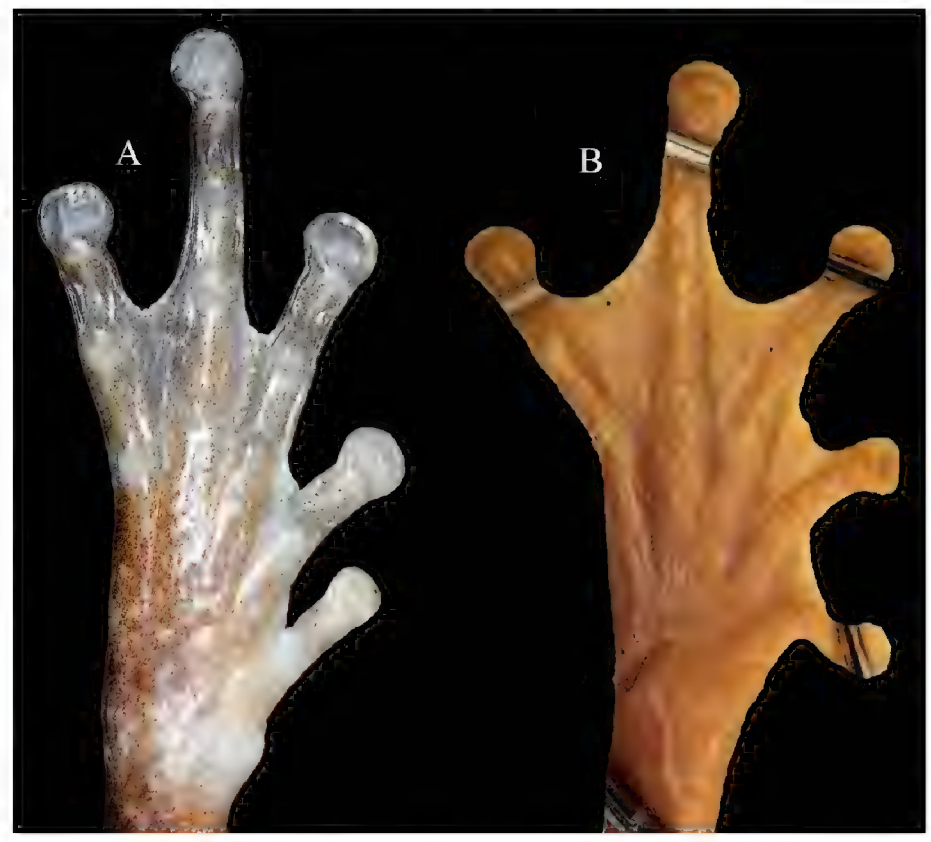

Figure 11. Ventral views of foot of *Gracixalus liusanjieae* sp. nov. (**A.** GXNU YU000796, photo by Guohua Yu) and *G. carinensis* (**B.** Reproduced from Matsui et al. 2017).

Gracixalus liusanjieae sp. nov. differs from *G. nonggangensis* by dorsal surface beige to brown with dark-brown mark (vs. yellowish-olive with dark-green mark), nuptial pads on fingers I and II (vs. absent), iris bronze (vs. olive), and advertisement calls composed of two notes (vs. 40–72 notes); from *G. patkaiensis* Boruah, Deepak, Patel, Jithin, Yomcha & Das, 2023 by bigger body size (SVL 27.5–31.5 mm in males vs. 23.6–26.5 mm in males), dorsal surface beige to brown (vs. green with brown spots),

vocal sac internal (vs. external), and nuptial pads on fingers I and II (vs. on finger I); from *G. quangi* Rowley, Dau, Nguyen, Cao & Nguyen, 2011 by larger body size (SVL 27.5–31.5 mm in males vs. 21.4–24.5 mm in males), dorsal surface beige to brown (vs. olive-green), vocal sac internal (vs. external), snout rounded (vs. triangularly pointed), nuptial pads present on fingers I and II (vs. on finger I), and tibiotarsal projection absent (vs. present); and from *G. quyeti* (Nguyen, Hendrix, Böhme, Vu & Ziegler, 2008) by dorsal surface beige to brown (vs. brownish to moss-green), head wider than long (vs. head longer than wide), tibiotarsal articulation reaching center of eye (vs. reaching to snout), supratympanic fold distinct (vs. indistinct), and forelimb, dorsal parts of thighs, tibia, and foot brown with dark brown bands (vs. moss-green with dark brown bands).

The new species differs from *G. sapaensis* Matsui, Ohler, Eto & Nguyen, 2017 by nuptial pads present on fingers I and II (vs. on finger I) and ventral surface of throat and chest creamy white (vs. light yellow); from *G. seesom* Matsui, Khonsue, Panha & Eto, 2015 by bigger body size (SVL 27.5–31.5 mm in males vs. 21.6–23.0 mm in males), snout rounded (vs. triangularly pointed), dorsal surface rough scattered with tubercles (vs. smooth), dorsal surface beige to brown with dark brown inverted Y-shaped marking (vs. tan), and nuptial pads present on fingers I and II (vs. absent); from *G. supercornutus* (Orlov, Ho & Nguyen, 2004) by bigger body size (SVL 27.5–31.5 mm in males vs. 22.0–24.1 mm), dorsal surface beige to brown (vs. dorsum green with brown spots), snout rounded (vs. pointed), white patch on temporal region absent (vs. present), dorsal surface nearly smooth (vs. considerable bigger horn-like projections in supraorbital area, around cloaca, and on dorsum, forelimbs and hindlimbs), and tibiotarsal projection absent (vs. present); and from *G. tianlinensis* by vocal sac internal (vs. external), ventral of thigh rough with tubercles (vs. ventral surface of limbs smooth), finger webbing rudimentary (vs. absent), and calls lacking harmonic (vs. present).

Gracixalus liusanjieae sp. nov. differs from *G. trieng* Rowley, Le, Hoang, Cao & Dau, 2020 by smaller body size (SVL in males 27.5–31.5 mm vs. 37.2–41.4 mm), presence of white tubercles on temporal region, edge of upper and lower jaw, flank, and dorsal surface of limbs (vs. absent), and throat and chest white with dark specks (vs. yellow or yellowish brown with pinkish mottling); from *G. truongi* Tran, Pham, Le, Nguyen, Ziegler & Pham, 2023 by smaller body size (SVL 27.5–31.5 mm in males vs. 32.2–33.1 mm in males), dorsal surface beige to brown (vs. moss green with grey), and nuptial pads present on fingers I and II (vs. on finger I); from *G. yunnanensis* Yu, Li, Wang, Rao, Wu & Yang, 2019 by vocal sac internal (vs. external), linea masculine absent (vs. present), and nuptial pads present on fingers I and II (vs. on finger I); and from *G. ziegleri* Le, Do, Tran, Nguyen, Orlov, Ninh & Nguyen, 2021 by nuptial pads present on fingers I and II (vs. on finger I), skin of throat relatively smooth (vs. granular), and tibiotarsal articulation reaching center of eye (vs. reaching tip of snout).

Discussion

The species diversity in the genus *Gracixalus* erected by Delorme et al. (2005) was underestimated. In recent years, a series of new species of this genus has been reported from China and Indochina, such as *G. guangdongensis* (Wang et al. 2018), *G. tianlinensis* (Chen et al. 2018), *G. yunnanensis* (Yu et al. 2019), *G. ziegleri* (Le et al. 2021), *G. patkaiensis* (Boruah et al. 2023), *G. trieng* (Rowley et al. 2020), *G. weii* (Liu et al. 2025), and *G. huaping* (Luo et al. 2025). Some of these recently described species (e.g. *G. yunnanensis*, *G. guangdongensis*, and *G. huaping*) have been confused with *G. jinxiuensis*, a species was once recorded widely in China, Vietnam, and Laos (Orlov et al. 2004; Nguyen et al. 2008; Shen 2014; Matsui et al. 2015, 2017; Rowley et al. 2011; Tran et al. 2023), implying that the species diversity of *G. jinxiuensis* species complex is underestimated and more studies are needed to clarify the taxonomic status of other records of *G. jinxiuensis* (Luo et al. 2025). Our finding of the new species *G. liusanjieae* sp. nov. from Mao'er Mountain, Guangxi further confirms this viewpoint and indicates that more efforts on the taxonomy of *G. jinxiuensis* species complex will likely result in further discoveries of yet unknown lineages.

Including the new species *G. liusanjieae* sp. nov., the genus *Gracixalus* now contains 23 species, 12 of which are distributed in China, and six in Guangxi (Fig. 1). Guangxi is a part of the Indo-Burma Biodiversity Hotspot and is second only to Yunnan in amphibian species diversity in China (Tang et al. 2023). In recent years, a series of new species and new records of amphibian and reptile were discovered in Guangxi, especially in its northeastern part that geographically belongs to Nanling Mountains area (e.g., Lyu et al. 2019; Chen et al. 2022; Pan et al. 2025). The Nanling Mountains are the largest mountain range as well as an important natural geographical boundary between southern and central subtropical China (Zhou et al. 2018). Serving as refugium, evolutionary museum, and geographic barrier for various floral and faunal taxa (López-Pujol et al. 2011; Gong et al. 2016; Tian et al. 2018; Hu et al. 2021; Mi et al. 2021; Wang et al. 2023) and recognized as a biodiversity hotspot, the region of Nanling Mountains has exceptional species richness and is characterized by exceptionally high levels of species endemism for animals (Wang et al. 2024; Xu et al. 2024). In the case of amphibian biodiversity in China, the hotspot of Nanling Mountains is second only to the hotspot of Southwest Yunnan Mountains (Xu et al. 2024). The Nanling Mountains are composed of a series of discontinuous mountains separated by basins, terraces, plains, and hills with low altitude (Miao et al. 2020), which might have promoted the divergence and speciation of species with low dispersal ability that adapted to relatively high altitude such as *Gracixalus* species in this region (*G. huaping*, *G. jinxiuensis*, and *G. liusanjieae* sp. nov.).

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Supplementary material 1

Genetic distance (uncorrected p-distance) between *Gracixalus* species estimated from 16S sequences

Authors: Xiangjian Wu, Yuanqiang Pan, Ju Chen, Jianping Ye, Guohua Yu, Tongxiang Zou

Data type: xls

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